METHODS OF TREATING HEART FAILURE AND FIBROTIC DISORDERS USING MODIFIED RELAXIN POLYPEPTIDES

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a divisional of U.S. Ser. No. 16/285,986, filed Feb. 26, 2019, which is a divisional of U.S. patent application Ser. No. 15/386,347, filed Dec. 21, 2016, now U.S. Pat. No. 10,253,083, which is a divisional of U.S. patent application Ser. No. 13/774,349, filed Feb. 22, 2013, now U.S. Pat. No. 9,567,386, which is a continuation-in-part of U.S. patent application Ser. No. 13/212,101, filed Aug. 17, 2011, now U.S. Pat. No. 8,735,539, which claims benefit to U.S. Provisional Pat. Appl. No. 61/374,582, filed Aug. 17, 2010, each of which is hereby incorporated by reference in its entirety.

SEQUENCE LISTING

This application includes a sequence listing which has been submitted via EFS-Web in a file named "11432700001210.txt" created May 26, 2020, and having a size of 54,295 bytes, which is hereby incorporated by reference in its entirety.

FIELD

The present disclosure relates to relaxin polypeptides and uses thereof. In exemplary embodiments, the relaxin polypeptides, such as human relaxin-2 polypeptides, may include one or more amino acid modifications and/or post-translational modifications, such as linkage to a polyethylene glycol or other half-life extending molecule. The disclosure further provides pharmaceutical compositions and medical use of such relaxin polypeptides.

BACKGROUND

Human relaxin 2 (RLX-2, H2-RLX, H2, RLXH2, bA12D24.1.1, bA12D24.1.2, hR2, RLN2) is a member of the insulin peptide family which includes the relaxins (RLX-1, RLX-2, RLX-3), insulin, and a number of insulin like peptides (INSL3-6), and the insulin-like growth factors (IGFI and IGFII) (Van Der Westhuizen et al., Curr Drug Targets, 2007. 8(1): p. 91-104). The active form of the protein consists of an A chain and a B chain linked by disulfide bonds. Relaxin is produced by the ovary, and targets the female reproductive system to ripen the cervix, elongate the pubic symphysis and inhibit uterine contraction. (Sherwood et al., Endocr Rev, 2004. 25(2): p. 205-34.)

Mature human relaxin is a hormonal peptide of approximately 6000 daltons known to be responsible for remodeling the reproductive tract before parturition, thus facilitating the birth process. This protein appears to modulate the restructuring of connective tissues in target organs to obtain the required changes in organ structure during pregnancy and parturition. See, Hisaw, F. L., Proc. Soc. Exp. Biol. Med., 23: 661-663 (1926); Schwabe, C., et al., Biochem. Biophys. Res. Comm., 75: 503-570 (1977); James, R. et al., Nature, 267: 544-546 (1977). A concise review of relaxin was provided by Sherwood, D. in The Physiology of Reproduction, Chapter 16, "Relaxin", Knobil, E. and Neill, J., et al. (eds.), (Raven Press Ltd., New York), pp. 585-673 (1988). Circulating levels of relaxin are elevated for the entire nine months of pregnancy and drop quickly following delivery.

While predominantly a hormone of pregnancy, relaxin has also been detected in the non-pregnant female as well as in the male (Bryant-Greenwood, G. D., Endocrine Reviews, 3: 62-90 (1982) and Weiss, G., Ann. Rev. Physiol., 46:43-52 (1984)) and has most recently been found to be useful in the treatment of heart failure.

Relaxin has been purified from a variety of species including porcine, murine, equine, shark, tiger, rat, dogfish and human, and shows at least primary and secondary structural homology to insulin and the insulin-like growth factor, however homology between species can be quite low. In the human, relaxin is found in most abundance in the corpora lutea (CL) of pregnancy. However, specific nuclei in the brain have relaxin receptors and other nuclei contain messenger RNA for relaxin. Several nuclei with cells bearing relaxin receptors are found in the area of the hypothalamus.

Two human gene forms have been identified, (H1) and (H2). Hudson, P., et al., Nature, 301: 628-631 (1983); Hudson, P., et al., The EMBO Journal, 3: 2333-2339 (1984); and U.S. Pat. Nos. 4,758,516 and 4,871,670. Only one of the gene forms (H2) has been found to be transcribed in CL. It remains unclear whether the (H1) form is expressed at another tissue site, or whether it represents a pseudo-gene. When synthetic human relaxin (H2) and certain human relaxin analogs were tested for biological activity, the tests revealed a relaxin core necessary for biological activity as well as certain amino acid substitutions for methionine that did not affect biological activity. Johnston, et al., in Peptides: Structure and Function, Proc. Ninth American Peptide Symposium, Deber, C. M., et al. (eds.) (Pierce Chem. Co. 1985).

A third relaxin gene form, Human relaxin-3 (also called H3 relaxin, RLX-3) is the equivalent to relaxin-2 in other species and is moderately conserved across species (amino acid homology of 77% between mouse and human) (Bathgate et al., J Biol Chem, 2002. 277(2): p. 1148-57). Because of its high expression levels in brain, human RLX-3 is considered a neuropeptide.

Methods of making relaxin are also described in U.S. Pat. No. 4,835,251 and in U.S. Pat. No. 5,464,756, international publication WO/1990/013659, U.S. Ser. No. 08/080,354, U.S. Pat. No. 5,759,807, and international publication no. WO/1995/000645. Methods of using relaxin in cardiovascular therapy and in the treatment of neurodegenerative diseases are described in U.S. Pat. No. 5,166,191 and in U.S. Ser. No. 07/902,637 (related to publication WO/1993/003755). Certain formulations of human relaxin are described in U.S. Pat. No. 5,451,572.

Recombinant human relaxin (H2) has been in Phase I human clinical trials in scleroderma patients. Scleroderma is a disease involving an imbalance in tissue reformation giving rise to the overproduction of collagen, and ultimately resulting in swelling and hardening of the skin (and affected organs). Currently treatments delivering relaxin require repeated and prolonged infusions.

Heart failure (HF) is defined as the inability of the cardiac pump to move blood as needed to provide for the metabolic needs of body tissue. Decreases in pumping ability arise most often from loss or damage of myocardial tissue. As a result, ventricular emptying is suppressed which leads to an increase in ventricular filling pressure and ventricular wall stress, and to a decrease in cardiac output. As a physiological response to the decrease in cardiac output, numerous neuroendocrine reflexes are activated which cause systemic vasoconstriction, sympathetic stimulation of the heart and fluid retention. Although these reflex responses tend to enhance cardiac output initially, they are detrimental in the